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Secondary metabolite variability in lichen genus *Usnea* in India: A potential source for bioprospection

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ABSTRACT

The species of lichen genus *Usnea* are well known for their worldwide distribution and medicinal properties due to presence of usnic acid. The genus is represented by more than 300 species in the world out of which 57 species are reported from India. In India, the species of *Usnea* grows profusely in higher altitudes of Western Ghats and Himalayan regions. 20 different secondary metabolites are known from Indian *Usnea* species which have potential biological and physiological significance. Salazinic acid, stictic acid, norstictic acid and barbatic acid are the most dominant secondary metabolites along with usnic acid. Apart from the natural thalli the cultivated mycobiont of *Usnea* species also produced many of these compounds in culture conditions. The secondary compounds present in *Usnea* species are significant source of natural products for commercial uses.

1) INTRODUCTION

Lichens are dual organism known to produce variety of secondary metabolites that act as defence molecules against physical stress and biological attacks during their slow growth in nature [1] and involved in maintaining symbiotic equilibrium [2, 3]. Being a symbiotic organism, the secondary metabolites produced by lichens are unique with respect to those of higher plants. The secondary metabolites in lichens are produced by mycobiont [4], that accumulate on extracellular surface of fungal hyphae.

Lichens have evolved metabolic pathways to synthesize secondary metabolites, mainly Acetyl-polymalonate pathway (APP), Shikimic acid pathway (SAP) and Mevalonic acid pathway (MAP). Most of the lichen compounds are phenolic compounds that come under orcinol/β-orcinol derivatives, dibenzofuranes, usnic acid, depsides (e.g. barbatic acid) & depsidones (e.g. salazinic acid). Due to the presence of useful metabolic compounds, lichens are in use for medicines, perfumes, dyes, cosmetics and as food from the ancient times. Many lichen secondary metabolites exhibit cytotoxic, antibiotic and antiviral properties and reported as potential source of pharmaceutically useful chemicals [5]. The species of lichen genus *Usnea* are most common among the different lichen groups used for mankind.

Lichen genus *Usnea* is globally distributed with more than 300 species [6]. In India, 57 species of *Usnea* are known which grow luxuriantly in higher regions of Western Ghats and Himalaya. The species of *Usnea* are known to be used in traditional medicines, in dyeing and in spices in various parts of the country. *U. longissima* Ach. has been widely used as expectorant, as a wound dressing and in the treatment of ulcers The 'Baiga', a primitive tribe of Madhya Pradesh used this species along with other ingredients for treating bone fracture [7]. In Unani literature the medicinal use of *Usnea* spp. are mentioned as astringent, antidote, analgesic, cardiotonic, resolvent and stomachic [8].

Since synthetic drugs cause numerous side effects and pathogenic micro-organisms develop antibiotic resistance, the natural compounds present in *Usnea* spp., are of great interest. In recent years, Malhotra et al. [9] evaluated the bioactivity of various extracts of *Usnea* species and recorded their role in discovery of new drugs. Santiago et al. [10] and Sultana & Afolayan [11] studied the antitumor activity, antioxidant activity and antimicrobial activity of *Usnea* spp., against various plants and animal pathogens. Rowe et al. [12] and Cocchietto et al., [13] studied the wide-spectrum antibiotic properties of *Usnea* spp. Singh et al. [14] reported that novel herbo-metallic preparation (nanoparticles) using extracts of U. longissima can inhibit bacterial quorum sensing. The isolated usnic acid has also been commercialized and is widely used as constituent of pharmaceutical preparations like herbal

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medicines and cosmetics. Apart from usnic acid the *Usnea* species also produced a number of beneficial bioactive secondary metabolites which are not so far identified, isolated and characterized.

The present study is aimed to list all the potent and useful secondary metabolites other than usnic acid, present in the Indian lichen species of *Usnea* and their biological activity so far known.

2) MATERIALS AND METHODS

More than 1500 specimens of lichen genus *Usnea* housed in herbarium of CSIR−National Botanical Research Institute , Lucknow (LWG) were studied. The morphological and anatomical characters of species were examined using Leica S8APO stereo zoom microscope and Leica DM 500 micro-system. The species were segregated using relevant keys and monographs [15, 16, 17]. The chemistry of the species was carried out using micro-crystallography and Thin Layer Chromatography (TLC) in solvent system A (180 ml toluene: 60 ml 1,4 dioxane: 8 ml acetic acid) and in solvent system C (85 ml Toluene: 15 ml acetic acid) following Orange et al. [18].

3) RESULTS AND DISCUSSION

The Indian species of lichen genus *Usnea* contain many biologically active secondary metabolites out of which only 21 compounds have been isolated and identified till now **(Table 1)**. Out of 57 *Usnea* species reported from the country, 12 species have more than one chemotypes. *U. longissima*, has maximum number of chemotypes as seven, followed by *U. dasaea* Stirt. and *U. subfloridana* Stirt which have five and four chemotypes respectively. Usnic acid, the most common secondary metabolite exhibits its presence in all the species of genus *Usnea*. The ecological function attributed to usnic acid is screening of excess light [19] as a result, species of *Usnea* growing on high altitudes could be potential source of high concentration of usnic acid. Apart from usnic acid, barbatic acid; norstictic acid; salazinic acid and stictic acids were the other commonly occurring lichen secondary metabolites in Indian *Usnea* species (**Figure 1**).

Alectorialic acid, barbatolic acid, ceparatic acid, cryptostictic acid, evernic acid, hypoconstictic acid, menegazziaic acid, protolichesterinic acid and thamnolic acid were among the rarely occurring lichen substances in Indian *Usnea* species. There were 14 species which contain unidentified spots of secondary metabolites. These unidentified spots of lichen substances in *Usnea* can be identified using advanced analytical tools in near future.

The secondary metabolites present in Indian *Usnea* species are derived from Acetate-Polymalonate Pathway (APP). These compounds can be broadly divided into two categories, i.e. aromatic products arising from inter-molecular estrification or oxidative coupling of orcinol/β-orcinol units and higher aliphatic acids. All the major secondary metabolites in *Usnea* belongs to aromatic products formed from β-orcinol units while ceparatic acid and protolichesterinic acid belong to higher aliphatic acids.

The chemical structures and useful biological activities of secondary metabolites present in Indian *Usnea* species have been documented in various research reports (**Table 2**). The bioactivity of lichen substances ranges from antimicrobial, antiviral, antitumor, antioxidant to analgesic, antipyretic and cardiovascular protective activities. The number of lichen secondary metabolites with specific biological activities is given in **figure 2**.

Majority of lichen substances present in Indian *Usnea* species have antimicrobial properties against plants and animal pathogens indicating that Indian *Usnea* species can be used as potential source of pharmaceutical medicines, herbal pesticides and microbicides.

Some lichen substances are antitumor agent while others are antioxidant in nature. Only few lichen substances of Indian *Usnea* species are reported to be analgesic, antipyretic and cardiovascular protective activities.

4) CONCLUSION

According to Culberson et al. [37] and Stocker-Worgotter et al. [38], the chemical variation in lichens seems to be genetically determined but the induction of biosynthetic pathways for production of secondary metabolites is also influenced by physiological and environmental factors. The unique micro-climatic conditions prevailing in Indian subcontinent makes it suitable habitat for chemically diverse species of lichens, like that of *Usnea*. The variable abiotic environmental factors of different geographic regions of the country influence the production of lichen substances in *Usnea* species resulting in high degree of chemotypes formation.

The chemical variability in Indian *Usnea* species can serve as source of valuable bio-products and are potential source of bio-prospection.

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Figure 1. Graph showing number of lichen secondary metabolites present in various Indian species of *Usnea*.

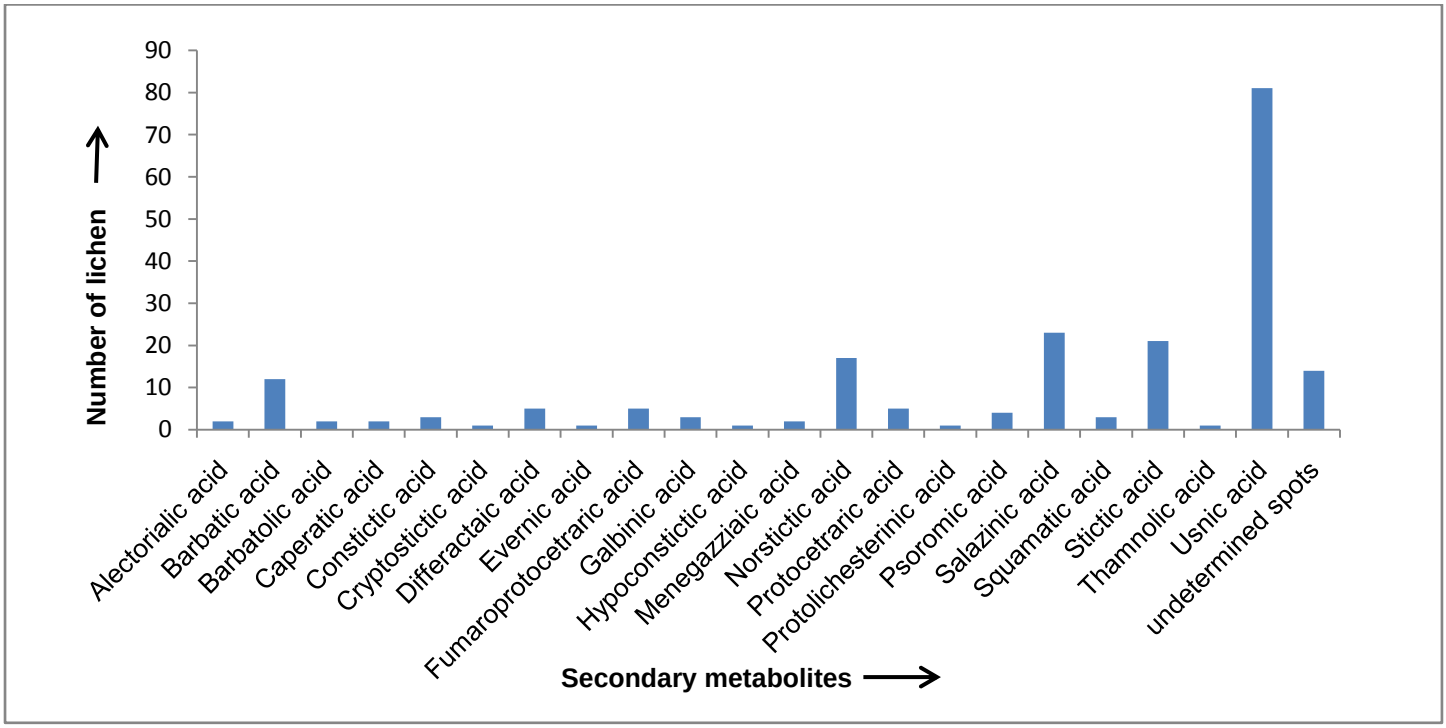
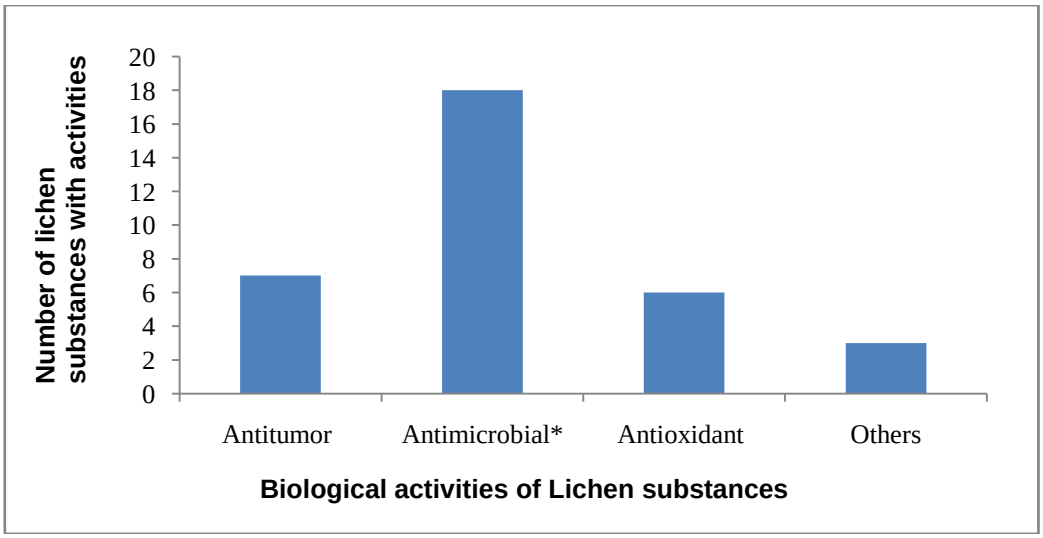


Fig. 2. Graph showing the number of lichen secondary metabolites showing different biological activities.



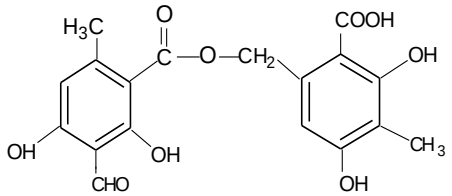
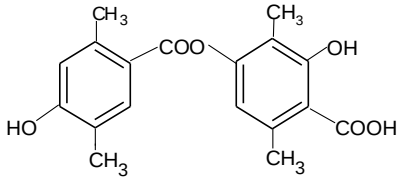
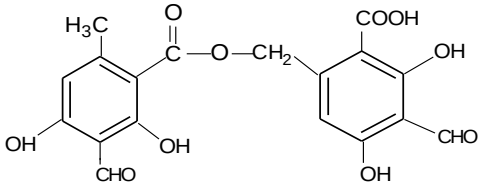
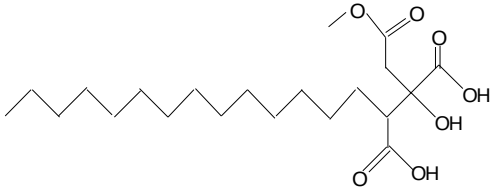
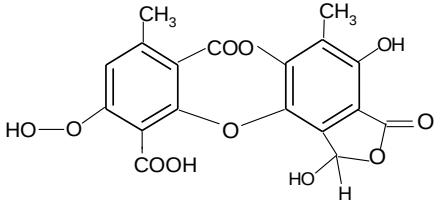
* Antimicrobial properties including antiviral properties

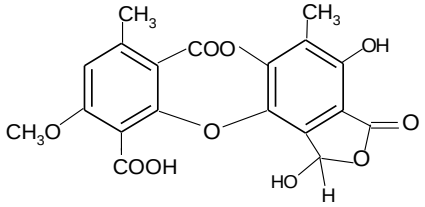
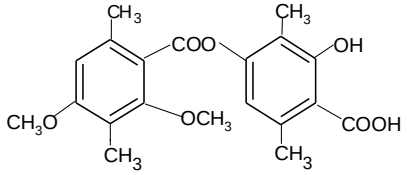
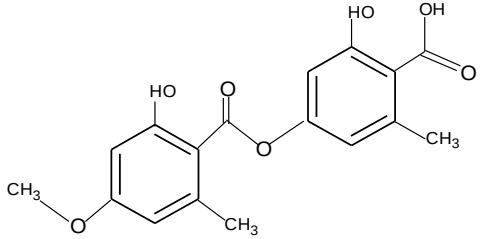
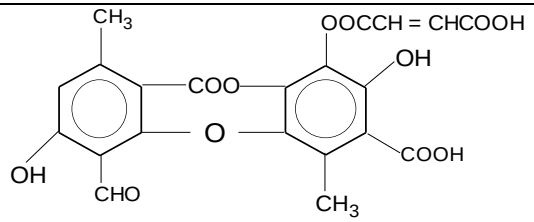
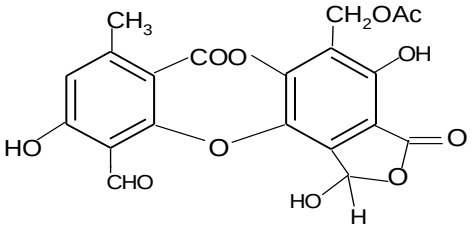
Table 1. The secondary metabolites present in Indian *Usnea* species. Ale= Alectorialic acid, Bar= Barbatic acid, Bat= Barbatolic acid, Cap= Caperatic acid, Con= Constictic acid, Cry= Cryptostictic acid, Dif= Differactaic acid, Evr= Evernic acid, Fum= Fumaroprotocetraric acid, Gal= Galbinic acid, Hyp= Hypoconstictic acid, Men= Menegazziaic acid, Nor= Norstictic acid, Pro= Protocetraric acid, Prl= Protolichesterinic acid, Pso= Psoromic acid, Sal= Salazinic acid, Sqa= Squamatic acid, Stc= Stictic acid, Thm= Thamnolic acid, Usn= Usnic acid, Und= undetermined spots.

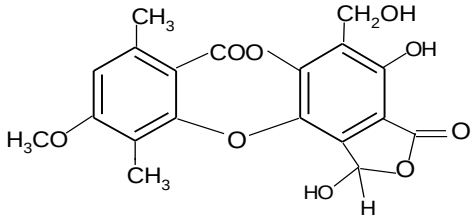
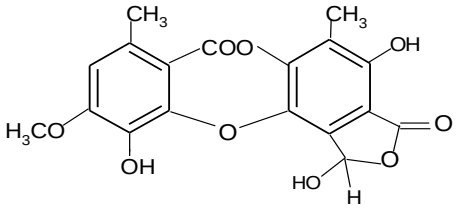
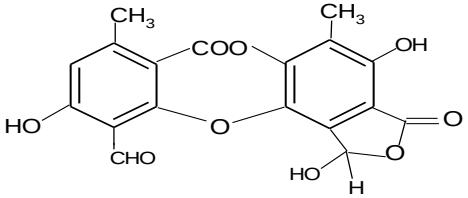
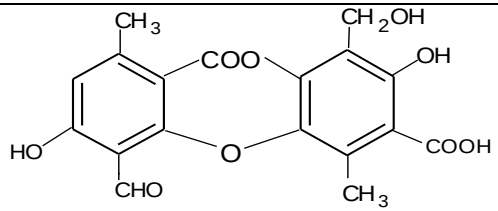
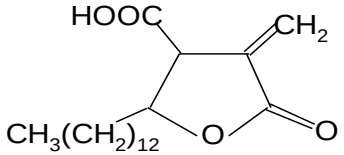
S. No.	Species	Ale	Bar	Bat	Cap	Con	Cry	Dif	Evr	Fum	Gal	Hyp	Men	Nor	pro	Prl	Pso	Sal	Sqa	Stc	Thm	Usn	Und
1.	*U. aciculifera* Vain.					+														+		+	
2.	*U. albopunctata* Nyl.															+						+	+
3.	*U. angulata* Ach.													+								+	
4.	*U. austro-indica* G. Awasthi (i) Strain I		+																			+	
	(ii) Strain II																					+	+
5.	*U.baileyi* (Stirt.) Zahlbr (i) Strain I									±				+				+				+	+
	(ii) Strain II													+								+	+
6.	*U. bismolliuscula* Zahlbr																			+		+	
7.	*U. bornmuelleri* J. Steiner																+					+	
8.	*U. certaina* Ach.							+														+	
9.	*U. complanata* (Muell. Arg.) Motyka (i) Strain I										+			+				+				+	
	(ii) Strain II																			+		+	
10.	*U. compressa* Taylor in Hook. f.																	+				+	
11.	*U. corallina* Mot.										+			+				+				+	
12.	*U. dasaea* Stirt. (i) Strain I																	+				+	+
	(ii) Strain II																+					+	
	(iii) Strain III										+	+	±	+				+				+	
	(iv) Strain IV																			+		+	
	(v) Strain V														±							+	
13.	*U. dendritica* Stirt.	+	+															+				+	
14.	*U. eumitrioides* Motyka																			+		+	
15.	*U. firmula* (Stirt.) Mot.														+							+	+
16.	*U. fischer*i G.Awasthi																			+		+	
17.	*U. fragilis* Stirt.		+																			+	

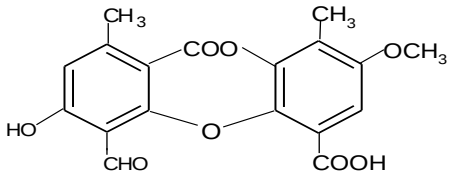
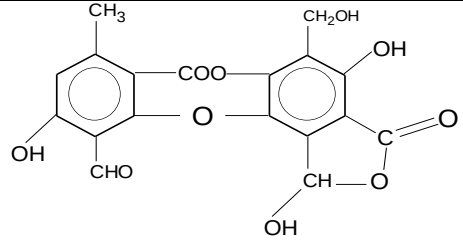
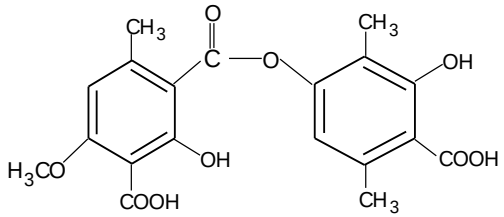
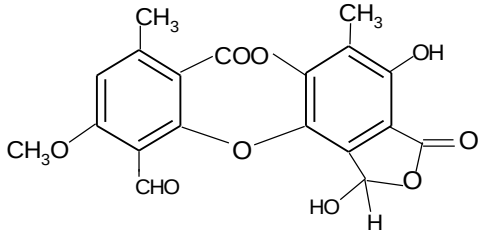
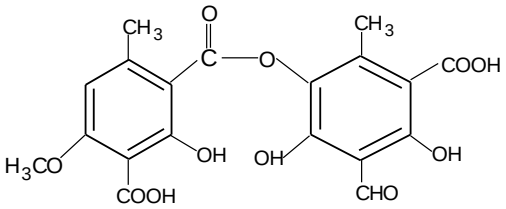
18.	*U. fulvoreagens* (Räsänen) Räsänen							+						+						+		+	
19.	*U. ghattensis* G. Awasthi																					+	+
20.	*U. himalayana* C. Bab. (i) Strain I													+								+	
	(ii) Strain II																	+				+	
	(iii) Strain III																			+		+	
21.	*U. himantodes* Stirt.																			+		+	
22.	*U. hirta* Mot.													+								+	
23.	*U. indica* Motyka																			+		+	
24.	*U. inermis* Mot.													±			+					+	
25.	*U. longissima* Ach. (i) Strain I																					+	
	(i) Strain II		+	+																		+	
	(i) Strain III		+	+															+			+	
	(i) Strain IV							+														+	
	(i) Strain V								+													+	+
	(i) Strain VI									+												+	+
	(i) Strain VII																		+			+	+
26.	*U. lucea* Motyka																			+		+	
27.	*U. luridorufa* Stirt.													+				+		+		+	
28.	*U. maculata* Stirt. (i) Strain I														+							+	
	(ii) Strain II																					+	
29.	*U. mekista* (Stirt.) G. Awasthi									+												+	
30.	*U. nepalensis* D.D. Awasthi in G. Awasthi																					+	
31.	*U. nilgirica* G. Awasthi		+							+												+	
32.	*U. nipparensis* Ashahina				+															±		+	
33.	*U. norketti* G.Awasthi		+															+				+	
34.	*U. orientalis* Mot.																	+				+	
35.	*U. pangiana* Stirt.		+					+										+				+	
36.	*U. pectinata* Taylor																			+		+	
37.	*U. perplexans* Stirt.																	+				+	
38.	*U. picta* (i) Strain I					±								±				+				+	
	(ii) Strain II																			+		+	

39.	*U. pictoides* G. Awasthi																				+	+	
40.	*U. pseudojaponica* G. Awasthi																			+		+	
41.	*U. pseudosinensis* Asahina		+													+						+	
42.	*U. rigidula* (Stirt.) G. Awasthi (i) Strain I																+					+	
	(ii) Strain II																+					+	+
	(iii) Strain III																			+		+	
43.	*U. robusta* Stirt.		±														+					+	
44.	*U. rubicunda* Stirt. (i) Strain I													+			+					+	
	(ii) Strain II																			+		+	
45.	*U. sinensis* Mot.																					+	
46.	*U. sordida* Mot.																+					+	
47.	*U. spinosula* Stirt.																			+		+	
48.	*U. splendens* Stirt.																+					+	
49.	*U. stigmata* Mot.					+	+						+	+						+		+	+
50.	*U. stigmatoides* G. Awasthi																			+		+	
51.	*U. subflorida* (Zahlbr.) Mot.		±												+							+	
52.	*U. subfloridana* Stirt. (i) Strain I													+								+	
	(ii) Strain II													±			+					+	
	(iii) Strain III																	+				+	
	(iv) Strain IV																				+	+	
53.	*U. thomsonii*	+						+														+	
54.	*U. trichodeoides* Vain., (i) Strain I									+												+	
	(i) Strain II														±		+					+	
55.	*U. vulneraria* Mot.				±									+			+					+	
56.	*Usnea* sp. 1		±																			+	+
57.	*Usnea* sp. 2																					+	+
	Total	**2**	**12**	**2**	**2**	**3**	**1**	**5**	**1**	**5**	**3**	**1**	**2**	**17**	**5**	**1**	**4**	**23**	**3**	**21**	**1**	**81**	**14**

S. No.	Secondary metabolites in *Usnea*	Structural information	Activity	References
1.	Alectorialic acid		Antiviral activity	Omarsdottir et al. [20]
2.	Barbatic acid		Antimicrobial activity, antiproliferative activity	Martins et. al. [21]; Kumar & Muller [22]
3.	Barbatolic acid		Antimicrobial activity	Cobanoglu et al. [23]
4.	Caperatic acid		Antioxidant activity	Ravaglia et al. [24]
5.	Constictic acid		Anticancerous activity	Shrestha et al. [25]

6.	Cryptostictic acid		Antimicrobial activity	Cobanoglu et al. [23]
7.	Differactaic acid		Antifungal agent, antiviral, antitumor, analgesic and antipyretic; inhibition of leukotriene B_4 biosynthesis (LTB) in leukocytes	Kumar & Muller [22, 26]
8.	Evernic acid		Fungicidal, Total/Strong growth inhibitor of plant pathogens; antimicrobial activity	Halama & van Haluwin [27], Manojlovic et al. [28]
9.	Fumaroprotocetraric acid		Antimicrobial activity	Rankovic et al. [29]
10.	Galbinic acid		Antibacterial activity	Sultana & Afolayan [11]

11.	Hypoconstictic acid		Antibacterial and antitumor activity	Sultana & Afolayan [11], Kwabena et al. [30]
12.	Menegazzaic acid		Antibacterial activity	Sultana & Afolayan [11]
13.	Norstictic acid		Antibacterial activity	Sultana & Afolayan [11]
14.	Protocetraric acid		Antimicrobial activity	Rankovic and Misic [29]
15.	Protolichesterinic acid		Antibacterial and antifungal activities, antiproliferative activity	Sasidharan, N.K. et al. [31], Hirayama et al. [32], Manojlovic et al. [28]

16.	Psoromic acid		Antioxidant and cardiovascular protective activities	Behera et al. [33]
17.	Salazinic acid		Antibacterial activity, antioxidant acitvity	Sultana & Afolayan [11], Amo De Paz et. al. [34]
18.	Squamatic acid		Antimicrobial activity	Manojlovic et al. [28]
19.	Stictic acid		Antimicrobial, anticancer activity and antioxidant acitvity	Rankovic and Misic [29], Amo De Paz et. al. [34], B. Pejin et al. [35]
20.	Thamnolic acid		Antimicrobial activity	Manojlovic et al. [28]

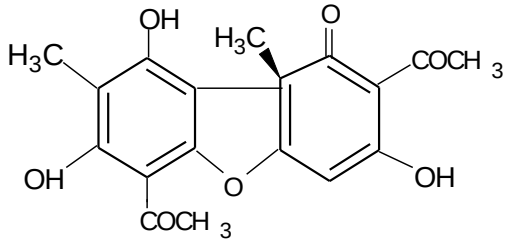
21.	Usnic acid		Antitumor, antimutagenic, antioxidant, analgesic, antipyretic, enzyme inhibitory, hepato-protective and antimicrobial activities	Sultana & Afolayan [11], Manojlovic et al. [28], Mayer et al. [36], Behera et al. [33]

Table 2. Details of secondary metabolites present in Indian *Usnea* species, their chemical structure, biological activities and references.